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Original article

Synthesis and Purification of 7-Prenyloxycoumarins and Herniarin as Bioactive Natural Coumarins

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Abstract

Objective(s)
7-prenyloxycoumarins including 7-isopentenyloxycoumarin, auraptene and umbelliprenin, and herniarin have been widely recognized as bioactive coumarins. This paper presents the ways to synthesis these compounds.

Materials and Methods
7-prenyloxycoumarins were synthesized by reaction between 7-hydroxycoumarin (1 M) and relevant prenyl bromides (1.5 M) in acetone at room temperature. The reaction was carried out in the presence of DBU (1, 8-diazabicyclo [5.4.0] undec-7-ene) (2 M). After 24 hr, the mixture was concentrated under reduced pressure. The compounds were purified by column chromatography.

Results
Three bioactive 7-prenyloxycoumarins, namely, umbelliprenin, auraptene and 7-isopentenyloxycoumarin, together with herniarin were synthesized from 7-hydroxycoumarin under alkaline conditions (DBU) and then purified by column chromatography. The structures of the products were characterized by NMR spectroscopic method including ^{1}H- and ^{13}C-NMR experiments.

Conclusion
The method of synthesis for 7-prenyloxycoumarins and herniarin which is presented here has not been reported yet. Moreover, for the first time, umbelliprenin was chemically prepared in this work.

Keywords: Auraptene, Herniarin, 7- Isopentenyloxycoumarin, 7-Prenyloxycoumarins, Synthesis, Umbelliprenin

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Introduction

7-Prenyloxycoumarins are a group of secondary metabolites which are found mainly in plants belonging to the families of Rutaceae and Umbelliferae. These compounds possess various biological and pharmacological activities which have gained attention of researchers in the last two decades. Three of the most studied prenylated coumarins include auraptene, umbelliprenin and 7-isopentenyloxycoumarin.

Umbelliprenin (Figure 1) is a naturally occuring prenylated coumarin which is synthesized by various *Ferula* species (1-4). It has also been found in various plant species consumed as food or used for food preparation such as in celery, *Angelica archangelica*, *Coriandrum sativum* and *Citrus limon* (5). It has been reported that umbelliprenin inhibits the red pigment production in *Serratia marcescens* (2), inhibits squalene-hopene cyclase (SHC) (an enzyme taking part in sterol synthesis) (6), decreases matrix metaloproteinase (MMP) activity (4), exhibits antileishmanial activity against promastigotes (3), induces apoptosis in human M4Beu metastatic pigmented melanoma cells (7) and exerts cancer chemopreventive activity (8). Auraptene (7-geranyloxycoumarin) is the most abundant geranyloxycoumarin extracted from plants belonging to the genus *Citrus* (1), and is known as a potent cancer chemopreventive (8-10) and anti-tumor agent against many types of cancers (9-13). Moreover, it exerts anti-inflammatory activity (14, 15) and is capable of suppressing the release of tumor necrosis factor alpha (TNF-α) (16), superoxide anion generation by inflammatory leukocytes (17) and I_KB (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor) degradation (18). Auraptene also causes complete inhibition of platelet aggregation, induced by arachidonic acid and platelet activated factor *in vitro* (19). Finally, auraptene and its analogs have been shown to exhibit spasmolytic activity (20, 21). 7-isopentenyloxycoumarin extracted from plants belonging to genus *Ruta* (1), has been shown to possess promising antifungal activity (22, 23). Besides, this compound exerts antitumor (23) and chemopreventive activity (24). Recently, auraptene and 7-isopentenyloxycoumarin have been found to possess significant neuroprotective activity (25). Herniarin occurs commonly in higher plants, e.g. *Matricaria* spp. (26, 27) and *Lavandula* spp. (28). In a previous report, herniarin was introduced to have anti-dermatophytic activity (29).

Angioni and colleagues (30) have reported the preparation of auraptene with a reaction between 7-hydroxycoumarin and geranyl bromide in K_2CO_3 solution. In another work, auraptene was synthesized from umbelliferone by prenylation with NaH and geranyl bromide in DMF (31). Herniarin was also prepared via a reaction between ortho-methoxy phenol and alkynoates in the presence of a palladium catalyst (32).

In the present study, we wish to report a new synthesis of four bioactive coumarins, namely, umbelliprenin, auraptene, 7-isopentenyloxycoumarin and herniarin, and their characterization, using NMR spectroscopic methods including ^{1}H- and ^{13}C-NMR spectroscopy.

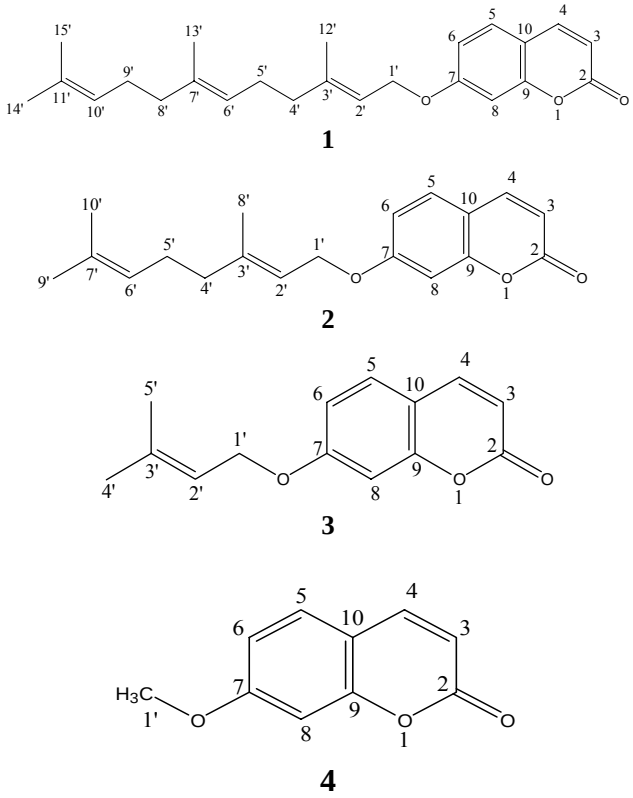

Figure 1. Chemical structures of 7-prenyloxycoumarins: umbelliprenin (1), auraptene (2) and 7-isopentenyloxycoumarin (3), and herniarin (4).

Materials and Methods

Chemistry

Melting points were determined on a Sanyo Gallenkamp apparatus. NMR spectra were measured on a Bruker DRX AV 500 (Bruker Biospin, Rheinstetten, Germany). [1] H- and 13C-NMR spectrum were measured, using an inverse-detection probe (5 mm). The operating frequencies were 500.13 MHz for acquiring ^{1}H-NMR and 125.75 MHz for ^{13}C-NMR spectra. Samples were measured at 300 K in $CDCl_3$ with TMS as the internal standard. Column chromatography was conducted with silica gel 230-400 mesh (Merck).

Preparation of umbelliprenin [(E, E)-7-(3, 7, 11-trimethyl-2, 6, 10-dodecatrienyloxy) chromen-2-one]

Umbelliprenin (7-farnesyloxycoumarin) was synthesized in 71% yield by reaction between 7-hydroxycoumarin (1 M) and *trans-trans*-farnesyl bromide (1.5 M) in acetone at room temperature. The reaction was carried out in the presence of DBU (1, 8-diazabicyclo [5.4.0] undec-7-ene) (2 M). After 24 hr, the mixture was concentrated under reduced pressure. Umbelliprenin was purified by column chromatography (petroleum ether/ethyl acetate 9:1 v/v) as white crystals (mp = 57.5-59.1 ºC) (5). The chemical structure of umbelliprenin is shown in Figure 1, and ^{1}H- and ^{13}C-NMR data for this compound are presented in Tables 1 and 2.

Preparation of auraptene [7-((E)-3, 7-Dimethylocta-2, 6-dienyloxy)-2H-chromen-2-one]

Auraptene (7-geranyloxycoumarin) was similarly synthesized in 65% yield by reaction between 7-hydroxycoumarin (1 M) and *trans*-geranyl bromide (1.5 M) in acetone at room temperature. The reaction was carried out in the presence of DBU (2 M). After 24 hr, the

Table 1. ^{1}H-NMR data for compounds **1-4** ($CDCl_3$, 500 MHz)[a].

H	1	2	3	4
2	-	-	-	-
3	6.25 d (9.6)	6.24 d (9.6)	6.24 d (9.6)	6.25 d (9.6)
4	7.63 d (9.6)	7.63 d (9.6)	7.63 d (9.6)	7.62 d (9.6)
5	7.36 d (7.2)	7.36 d (7.2)	7.36 d (7.2)	7.37 d (7.2)
6	6.85 dd (7.2, 2.0)	6.85 dd (7.2, 2.0)	6.85 dd (7.2, 2.0)	6.85 dd (7.2, 2.0)
7	-	-	-	-
8	6.82 d (2.0)	6.82 d (2.0)	6.82 d (2.0)	6.82 d (2.0)
9	-	-	-	-
10	-	-	-	-
1'	4.60 d (7.0)	4.59 d (7.0)	4.57 d (7.0)	3.86 d (7.0)
2'	5.47 t (7.0)	5.47 t (7.0)	5.47 t (7.0)	-
3'	-	-	-	-
4'	2.12 m	2.13 m	1.80 m	-
5'	2.15 m	2.15 m	1.76 m	-
6'	5.07 q (7.0)	5.08 q (7.0)	-	-
7'	-	-	-	-
8'	1.97 m	1.75 m	-	-
9'	2.05 m	1.65 m	-	-
10'	5.09 q (7.0)	1.59 q (7.0)	-	-
11'	-	-	-	-
12'	1.76 s	-	-	-
13'	1.60 s	-	-	-
14'	1.67 s	-	-	-
15'	1.59 s	-	-	-

[a] J values are in parenthesis and reported in Hz; chemical shifts are given in ppm.

mixture was concentrated under reduced pressure. Auraptene was easily purified by column chromatography (petroleum ether/ethyl acetate 9:1 v/v) as white crystals (mp = 62.7-63.4 ºC) (5). The chemical structure of auraptene is shown in Figure 1, and ^{1}H- and ^{13}C-NMR data for this compound are presented in Tables 1 and 2.

Table 2. ^{13}C-NMR data for compounds 1-4 ($CDCl_3$, 125.7 MHz).

C	1	2	3	4
2	161.2	161.2	161.2	161.1
3	112.9	112.9	112.9	112.5
4	143.4	143.4	143.4	143.3
5	128.6	128.6	128.6	128.7
6	113.2	113.2	113.1	113.0
7	162.1	162.1	162.0	162.8
8	101.5	101.5	101.5	100.8
9	155.8	155.8	155.8	155.8
10	112.4	112.3	112.3	112.5
1'	65.4	65.4	65.3	55.7
2'	118.4	118.3	118.5	-
3'	142.3	142.3	139.2	-
4'	39.5	39.4	25.7	-
5'	26.1	26.4	18.2	-
6'	123.4	123.5	-	-
7'	135.5	131.9	-	-
8'	39.6	16.7	-	-
9'	26.6	25.6	-	-
10'	124.2	17.6	-	-
11'	131.3	-	-	-
12'	16.7	-	-	-
13'	16	-	-	-
14'	25.6	-	-	-
15'	17.6	-	-	-

Preparation of 7-isopentenyloxycoumarin [7-(isopentenyloxy) chromen-2-one]

7-isopentenyloxycoumarin was also synthesized in 45% yield by reaction between 7-hydroxycoumarin (1 M) and isopentenyl bromide (1.5 M) in acetone at room temperature. The reaction was carried out in the presence of DBU (2 M). After 24 hr, the mixture was concentrated under reduced pressure. 7-isopentenyloxycoumarin was purified by column chromatography (petroleum ether/ethyl acetate 9:1 v/v) as white crystals (mp = 74.3-75.2 ºC) (5). The chemical structure of 7-isopentenyloxycoumarin is shown in Figure 1, and ^{1}H- and ^{13}C-NMR data for this compound are presented in Tables 1 and 2.

Preparation of herniarin (7-methoxychromen-2-one)

Herniarin (7-methoxycoumarin) was synthesized in 54.8% yield by reaction between 7-hydroxycoumarin (1 M) and methyl iodide (1.5 M) in acetone at room temperature. The reaction was carried out in the presence of DBU (2 M). After 24 hr, the mixture was concentrated under reduced pressure. Herniarin was purified by column chromatography (petroleum ether/ethyl acetate 9:1 v/v) as white crystals (mp= 120.0-121.0 ºC) (5). The chemical structure of herniarin is shown in Figure 1, and ^{1}H- and ^{13}C-NMR data for this compound are presented in Tables 1 and 2.

Results and Discussion

To our knowledge, the method of synthesis presented in the current study has not been reported to date, especially for umbelliprenin that has not been previously chemically prepared. However, Angioni and colleagues (30) reported the preparation of auraptene with a reaction between 7-hydroxycoumarin and geranyl bromide in K_2CO_3 solution (acetone). The yield of the mentioned reaction was 85% after 10 hr. In another work, auraptene was

synthesized from umbelliferone by prenylation with NaH and geranyl bromide in DMF (31). Trost and colleagues (32) recently reported a new method for the synthesis of herniarin and other coumarins in the presence of palladium catalyst. In our work, we studied the prenylation of 7-hydroxycoumarin with different prenyl moieties (isopentenyl, geranyl and farnesyl) by, using DBU as an alkaline reagent in acetone. The yield of each reaction is reported in the experimental section. Besides, the synthesis of herniarin as a non-prenylated coumarin has also been reported in this paper.

The ^{1}H- and ^{13}C-NMR data, together with melting points of the four synthesized compounds namely auraptene, umbelliprenin, 7-isopentenyloxycoumarin and herniarin were in agreement with those previously described in the literature (5, 33, 34).

The ^{1}H-NMR spectrum of umbelliprenin showed resonances characteristic for four methyl singlets at δ_H 1.59 (H-15'), 1.60 (H-13'), 1.67 (H-14') and 1.76 (H-12'), and eight methine resonances at δ_H 5.07 (H-6', t, J = 7.0 Hz), 5.09 (H-10', t, J = 7.0 Hz), 5.47 (H-2', t, J = 7.0 Hz), 6.25 (H-3, d, J = 9.6 Hz), 6.82 (H-8, d, J = 2.0 Hz), 6.85 (H-6, dd, J = 7.2 Hz, J = 2 Hz), 7.36 (H-5, d, J = 7.2 Hz) and 7.63 (H-4, d, J = 9.6 Hz). Three aromatic protons at δ_H 6.82 (H-8), 6.85 (H-6) and 7.36 (H-5) suggested the presence of a 7, 9, 10-trisubstituted benzene ring, which was supported by the ^{13}C-NMR spectrum.

The ^{13}C-NMR resonances displayed 24 carbon signals, nine being typical of an umbelliferone skeleton and the other 15 signals were ascribable to a prenyl (farnesyl) moiety. The ^{13}C-NMR spectrum showed four methyl signals at δ_C 16.0 (C-13'), 16.7 (C-12'), 17.6 (C-15') and 25.6 (C-14'), three methylene signals at δ_C 26.1 (C-5'), 26.6 (C-9') and 65.4 (C-1'), eight methine signals at δ_C 101.5 (C-8), 112.9 (C-3), 118.4 (C-2'), 113.2 (C-6), 124.2 (C-10'), 123.4 (C-6'), 128.6 (C-5) and 143.4 (C-4), and seven quaternary carbon signals at δ_C 112.4 (C-10), 131.3 (C-11'), 135.5 (C-7'), 155.8 (C-9), 142.3 (C-3'), 161.2 (C-2) and 162.1 (C-7), including one carbonyl function which was indicated by the downfield signal at δ_C 161.2 (C-2). These ^{1}H- and ^{13}C-NMR data were in accordance with those previously reported for umbelliprenin (5, 33).

The ^{1}H- and ^{13}C-NMR spectra of other coumarins including auraptene, 7-isopentenyloxycoumarin and herniarin were in agreement with the literature (34). Their structures were similarly characterized. The full NMR data of synthesized coumarins are shown in Table 1.

Conclusion

7-prenyloxycoumarins are easily synthesized via a reaction between prenyl bromides and umbelliferone in an alkaline condition (DBU in acetone) and then are successfully purified with silica column chromatography. Although, no by products were detected in the reaction, the reaction was not accomplished after 48 hr. It might be due to impurities of the reagents or other factors.

Finally, it is worth noting that the method of synthesis for 7-prenyloxycoumarins and herniarin which is presented here has not been reported yet. Moreover, for the first time umbelliprenin was chemically prepared in the present work.

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